outstanding Office Action in the above-identified patent application. As noted in the Examiner Interview Summary Record dated May 19, 1994, all rejections under 35 U.S.C. §102/103, for both the Shimokado and Campochiaro references have been withdrawn. Applicants wish to thank the Examiner for her time and helpful suggestions during the telephone interview. Applicants respectfully request reconsideration of the present application.

THE INVENTION

The present invention discloses a previously unknown factor called Connective Tissue Growth Factor (CTGF), which is related immunologically and biologically to Platelet Derived Growth Factor (PDGF). Various cell types produce and secrete PDGF and PDGF-related molecules. In an attempt to identify the type of PDGF dimers present in the growth media of cultured endothelial cells, a new growth factor was discovered.

CTGF appears to play a role in the development, growth and repair of normal human tissue. The discovery of the CTGF protein and cloning of the cDNA encoding this molecule is significant in that it is a previously unknown growth factor having mitogenic and chemotactic activities in connective tissue cells. Although the biological activity of CTGF is similar to that of PDGF, CTGF is the product of a gene unrelated to the A or B chain genes of PDGF.

Since CTGF is produced by endothelial and fibroblast cells, both of which are present at the site of a wound, it is believed that CTGF functions as a growth factor in wound healing. Pathologically, CTGF may be involved in diseases in which there is an overgrowth of connective tissue cells, such as cancer, fibrotic diseases and atherosclerosis.

The primary biological activity of CTGF polypeptide is its mitogenicity, or ability to stimulate target cells to proliferate. The ultimate result of this mitogenic activity *in vivo*, is the growth of target tissue. CTGF also possesses chemotactic activity, which is the chemically induced movement of cells as a result of interaction with particular molecules. The CTGF of this invention is mitogenic and chemotactic for connective tissue cells, however, other cell types may be responsive to CTGF polypeptide as well.

The CTGF polypeptide of the invention is characterized by existing as a monomer of approximately 36-38 kD molecular weight which is secreted by cells and is active upon interaction with a PDGF receptor in cells.

The Applicants successfully isolated and purified CTGF protein and cloned the nucleotide sequence encoding CTGF, both of which were previously unknown in the prior art.

REJECTIONS ON PRIOR ART

Claim 1 stands rejected under 35 U.S.C. §102(b) as anticipated by, or in the alternative under 35 U.S.C.§103 as obvious over Matsuoka, *et al.*, or alternatively Campochiaro, *et al.*, or alternatively, Shimokado, *et al.* Applicant respectfully traverses the rejection.

As indicated in the remarks, both the Campochiaro and Shimokado references have been withdrawn and therefore the rejection is moot with respect to these references. Matsuoka, *et al*. disclose a family of PDGF-related proteins found in human wound fluid. These proteins were identified using a polyclonal antiserum to PDGF. Two peptide fractions, from 16-17 kD and 34-36 kD, were found to be immunoreactive with the polyclonal antibodies. The Office Action states that the 34-36 kD protein fraction appears to be identical to the CTGF of the present invention.

The mitogenic and chemotactic activities in Matsuoka correlated only with the 16-17 kD peptide(s), as shown in Figure 4, page 4419. The 34-36 kD fraction possessed no biological activity and was only present in trace amounts at the time when PDGF-like bioactivity was observed in wound fluid. Page 4418, column 2, lines 15-27, describes the correlation between the peptides described in the reference and biological activity. The kinetics of appearance and disappearance of the 16-17 kD and 34-36 kD product were independent of each other. According to the analysis of Figure 4B and 4C on page 4418, the Figure legend is mislabeled; the closed circles represent the total wound fluid and the open circles represent the immunopurified material. Importantly, as stated in the discussion on page 4418, column 2, lines 15-27, the level of the 16-17 kD peptide peaked on the first day after surgery and decreased exponentially to nearly undetectable levels by the seventh day. In contrast, the 34-36 kD product was initially present at low levels and then increased, reaching peak levels on the fifth and sixth days postsurgery. The chemotactic and mitogenic activity of the total wound fluid and immunoabsorbed fraction show the highest activity on day 1 and decreased to undetectable levels by day 4, which correlates with the kinetics of appearance and disappearance of the 16-17 kD peptide.

Additional evidence that the 34-36 kDa fraction does not contain mitogenic activity is shown in the accompanying declaration under 37 CFR §1.132 by Dr. Gary Grotendorst and the accompanying exhibits. The experiments shown were perfomed in his laboratory under his supervision prior to the date of the Matsuoka reference (prior to June, 1989). The first experiment shows that the mitogenic activity present in the anti-PDGF IgG absorbed fraction after day 3 post surgery, at a time when the 34-36 kDa fraction is elevated, is the same as the negative control background indicating that there is no activity in the 34-36 kDa sample (SEE EXHIBIT A).

Briefly, anti-PDGF IgG purified wound fluid samples from days 1-5 post surgery were analyzed for mitogenic activity in a standard [^{3}H]-thymidine uptake assay for DNA synthesis (see Matsuoka, *et al.*, for experimental methods). The cpm in the day 1 sample (see #30, 17374 cpm) is significantly above the background level of 2226 to 3886 cpm in samples #51-54. However, the samples from days 3-5 (#33-38), which is when the 34-36 kDa fraction peaks (See Figure 4 of Matsuoka), range from 1735 to 3671, which is essentially the same as background. Therefore, the fraction which correlates with the appearance of the 34-36 kDa peak in Matsuoka, *et al.*, does not exhibit any mitogenic activity. (Samples 43-58 are standards containing different amounts of PDGF or, 49-50, FGF).

A second experiment examined chemotactic activity in the samples and indicated that none of the activity after day 3 could be neutralized with anti-PDGF IgG (SEE EXHIBIT B and Materials and Methods in Matsuoka, *et al.*). This indicates that the low level of activity is likely due to non-specific activation and that the PDGF-related 34-36 kDa protein fraction is not active as a chemoattractant.

Briefly, the ability of anti-PDGF IgG to neutralize the chemotactic activity present in the anti-PDGF IgG purified fraction (PDGF related) of human wound fluids collected on various days (D1=day 1, D2=Day 2, etc.) was examined. As seen in the column to the far right, only D1 shows significantly high activity which is able to be neutralized with anti-PDGF IgG. For example, sample 1 is Day 1 in the presence of non-immune IgG and activity is 157; sample 2 is the same material in the presence of anti-PDGF IgG had an activity of 28. Sample 19 is a 10 ng/ml PDGF standard in the presence of non-immune IgG and activity is 167; sample 20 is the same material in the presence of anti-PDGF IgG with an activity of 40. Therefore, samples with activity below 28-40 have no PDGF-like activity. The 16-17 kDa fraction shown in Figure 4 of Matsuoka peaks on Day 1 post surgery, the time at which chemotactic activity is the highest (see D1). In contrast, at a time when the 34-36 kDa fraction peaks (day 4-5), there is no detectable chemotactic activity.

Therefore, the biological activity found in Matsuoka, *et al.*, correlates with a 16-17 kD protein and not a 34-36 kD protein. In view of the failings of Matsuoka to teach or suggest the mitogenic, chemotactic polypeptide of the invention, Applicants respectfully request that the rejections based on this reference be withdrawn.

Claim 1 stands rejected under 35 U.S.C.§102(a) as anticipated by or, in the alternative under 35 U.S.C.§103 as obvious over Ryseck, *et al*. Applicants respectfully traverse this rejection.

As stated in the previous Amendment, the clone and sequence of CTGF were obtained by Applicants in the United States and submitted to GenBank on July 17, 1990, prior to the May 1991 publication date of Rysek. The Office Action states that a comparison of the amino acid sequence of fisp-12 and CTGF reveals only 13 discrepancies in the region between 86 to 392 and that there is greater divergence

in the region preceeding residue 86. The Office Action states that Ryseck identifies this region as a signal sequence which would not affect protein activity.

Applicants disagree with the conclusions stated in the Office Action. It is well known in the art that a typical signal sequence is about 15-25 amino acids in length. In fact, on page 227 of Ryseck, line 5, the authors state that the signal sequence of fisp-12 is only 21 amino acids (also see FIGURE 3). The cleavage site for the signal sequence is between residues 25 and 26 (page 226, column 2, second paragraph). Therefore, the sequence divergence found in amino acids 26-86 is significant and therefore the fisp-12 protein described by Ryseck is distinguishable from CTGF of the present invention.

Further, as shown in the accompanying declaration under 37 CFR §1.132 by Dr. Gary Grotendorst, prior to the May, 1991 date of the Ryseck reference, Applicants had immunoaffinity purified CTGF and shown that it had mitogenic activity in a DNA synthesis assay using NRK fibroblasts. EXHIBIT C shows laboratory notebook pages from Applicants (in Dr. Grotendorst's laboratory and under his supervision) for experiments which were performed prior to the date of the Ryseck reference showing that immunoaffinity purified CTGF has mitogenic activity.

Briefly, serum free (S/F) media from cultured HUVE cells was affinity purified on a column of Affi-Gel-10 conjugated with anti-PDGF IgG by methods described in Matsuoka, *et al*. (cited in this Office Action). Affinity purified material was analyzed in a Western blot and in a mitogenic assay using NRK cells as described by Matsuoka, *et al*. The data shown in EXHIBIT C, page 2 (table of cpm/sample) indicate that the affinity purified material (see for example samples 7-13) had mitogenic activity comparable to purified PDGF (samples 14-17).

Western blot analysis of the affinity purified mitogenic fractions revealed a protein with mitogenic activity that migrated at about 36 kDa. This protein fraction was identified as CTGF.

The identification of PDGF-like activity in HUVE cell conditioned media prompted the cloning and the isolation of a full length CTGF clone from a HUVE cell library (see Examples of the present patent application). The clone, designated DB60, was isolated from a HUVE cell cDNA library in λgt11 screened with anti-PDGF antibody (EXHIBIT D) prior to the date of Ryseck, *et al*. Anti-PDGF antibody binding to the fusion protein produced by the clone DB60 was completely blocked by the affinity purified proteins. Therefore, these experiments showed that the clone that was isolated encoded the chemotactic, mitogenic protein called CTGF. A Northern blot analysis using RNA from HUVE cells indicated that the clone hybridized with a mRNA of about 2.4 kb, which is a message of sufficient size to produce a protein in the 38 kD molecular weight range as seen on the immunoblots of the affinity purified proteins.

The clone encoding the entire open reading frame of the mitogenic and chemotatic CTGF protein was isolated prior to the May, 1991 date of the Ryseck reference in Dr. Grotendorst's lab and under his supervision.

In light of the comments above, it is respectfully requested that the Ryseck reference be withdrawn.

Claims 14-16 stand rejected under 35 U.S.C.§103 as allegedly unpatentable over Matsuoka, *et al*., or alternatively Campochiaro, *et al*., or alternatively over Shimokado, *et al*., or alternatively over Ryseck *et al*. Applicants respectfully traverse this rejection.

As stated above, both the Campochiaro and Shimokado references have been withdrawn and therefore the rejection is moot with respect to these references. In addition, as stated in the comments above, the CTGF of the present invention and the protein fraction in Matsuoka, or the nucleotide sequence identified in Ryseck, *et al.* are distinguishable. Antibodies which specifically bind CTGF and not PDGF could not have been produced prior to Applicants' discovery of the CTGF which is required for immunization. As stated above, the references fail to teach or disclose CTGF as defined in the present invention. Therefore, since the CTGF of the present invention is a novel protein, antibodies which specifically bind to CTGF must also be novel. The antibodies used for isolating the proteins in the above-cited references were raised against PDGF and not CTGF. Those antibodies are not specific for CTGF as they recognize other growth factors, including PDGF AA, AB and BB and leukocyte-derived growth factor (LDGF). Therefore, the antibodies of the invention which bind CTGF and not PDGF are novel.

There are also several fundamental problems with the Office Action's suggestions that it would be routine to isolate and purify the protein of the present invention from a fraction of proteins having mitogenic and chemotactic activity as described in the accompanying declaration by Dr. Gary Grotendorst. First, to purify the protein and determine its amino acid sequence would require at least 2-5 nanomoles of pure protein. For CTGF, this would require 72-180 μg based on a molecular weight of 36 kDa (36 μg=1 nm). The typical recovery of pure PDGF from platelets during purification is 10% or less of the total PDGF present in the starting material. This would require enough wound fluid or conditioned media that contained 720-1800 μg of CTGF. If the concentration present is less than 5 μg/liter, it would require 200-600 liters of wound fluid to isolate the protein. Typically less than 50 mls of wound fluid could be collected from a single patient so that 20 patients would be required for 1 liter of fluid and over 4000 patients would

be required for the collection of a minimal amount of fluid to purify the factor. It would require a nationwide effort to collect that amount of fluid and would be virtually impossible to accomplish.

The Applicants respectfully request that any rejections based on Matsuoka, *et al.* and Ryseck, *et al.* be withdrawn.

In summary, based on Applicants' comments above, it is respectfully submitted that claims 1 and 14-16 clearly and patentably define the invention. Applicants respectfully request that the Examiner reconsider the various grounds of rejection set forth in the Office Action and, in light of Applicants' response, allow the claims now pending to proceed to issuance.

Respectively submitted,

SPENSLEY HORN JUBAS & LUBITZ

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